

# **CoBOP: MICROBIAL BIOFILMS: A PARAMETER ALTERING THE APPARENT OPTICAL PROPERTIES OF SEDIMENTS, SEAGRASSES AND SURFACES.**

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## **LONG-TERM GOAL**

The long-term goal of my research is to understand the optical properties of microbial biofilms, which form coatings on sediments and other surfaces in coastal oceans. The specific project goals are to determine how biofilm coatings may influence (i.e., alter) optical spectra of sediments and other surfaces through reflection, scattering and fluorescence. This project is a part of the CoBOP (Coastal Ocean Benthic Optical Properties) initiative in the Environmental Optics Program.

## **OBJECTIVES**

The objective of year six was to perform data analyses with CoBOP Optics investigators. Our specific objectives were to: (1) conduct a coordinated laboratory experiment (with several CoBOP investigators) involving sediment/ exopolymer manipulations to determine how spectral reflectance may be altered by the presence of exopolymers and other sediment components; and (2) continue analyses of the microstructure of surface sediments using confocal scanning laser microscopy (CSLM); and (3) analyse and interpret existing field- and new laboratory-data to further understand the potential effects of biofilm exopolymers on altering sediment characteristics, and resulting changes in optical signatures.

## **APPROACH**

The results of manipulative field and laboratory experiments, (conducted at Lee Stocking island, and the Rosenstiel School of marine and atmospheric Sciences (RSMAS)) were examined in collaboration with several CoBOP personnel (E. Louchard, Drs. R.P. Reid, and C. Stephens-RSMAS (U. Miami); K. Voss (U. Miami) R. Wheatcroft –U. Oregon; and M. Allison (Tulane Univ.) and C. Mazel (PSI Corp).

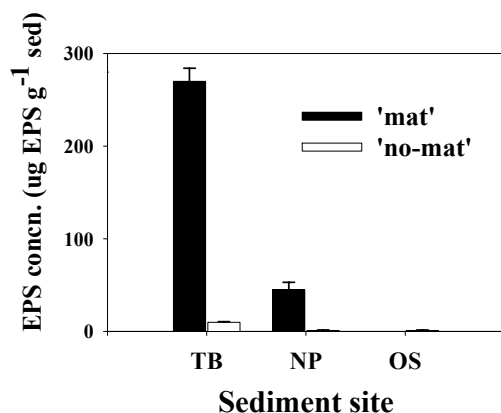
## **WORK COMPLETED**

The combined results of our fieldwork, laboratory experiments, and data analyses have been highly successful. Measurements of spectral reflectance, porosity, in-sediment light fields, sediment surface light scattering and refractive index measurements, were conducted. Quantitative imaging of *in-situ* sediment, generated by nanoplast-embedded natural sediments, was generated by scanning confocal laser microscopy.

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14. ABSTRACT <b>The long-term goal of my research is to understand the optical properties of microbial biofilms, which form coatings on sediments and other surfaces in coastal oceans. The specific project goals are to determine how biofilm coatings may influence (i.e., alter) optical spectra of sediments and other surfaces through reflection, scattering and fluorescence. This project is a part of the CoBOP (Coastal Ocean Benthic Optical Properties) initiative in the Environmental Optics Program.</b>					
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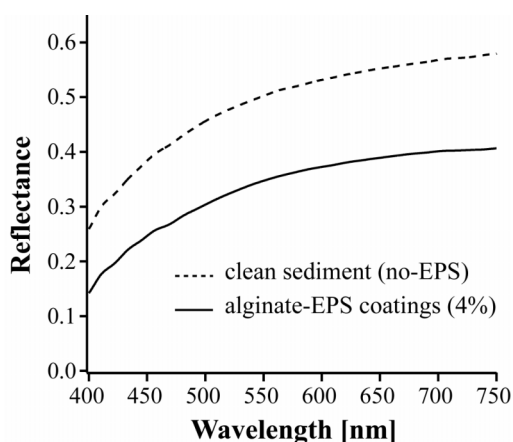
## RESULTS

A wide variation in exopolymer (EPS) concentrations occurs among the sediment sites. Within each site, EPS concentrations are significantly higher in “mat” sediments (i.e. having microbial mats on sediment surface) (Fig. 1).



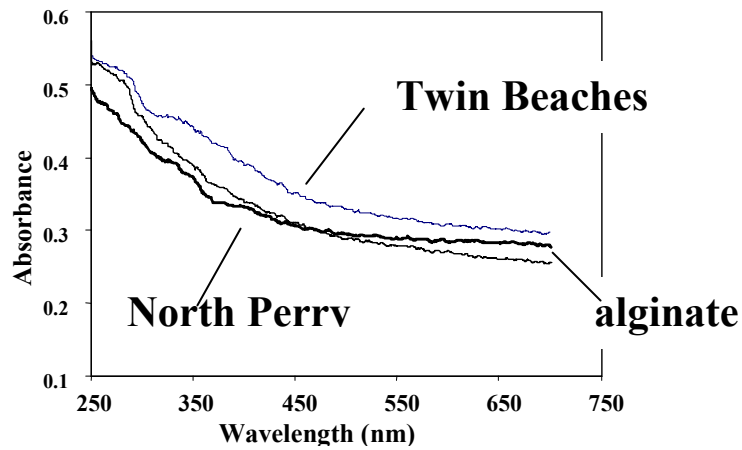
**Fig. 1. Exopolymer Abundances in natural marine sediments: Exopolymer concentrations in surface sediments are significantly higher when microbial mats are present.**

Our previous CoBOP work has shown that “mat” sediments have lower reflectance magnitudes compared with comparable sediments having “no mats”. Laboratory manipulative experiments showed that this decrease (in magnitude) is due to the presence of exopolymer (Fig. 2).



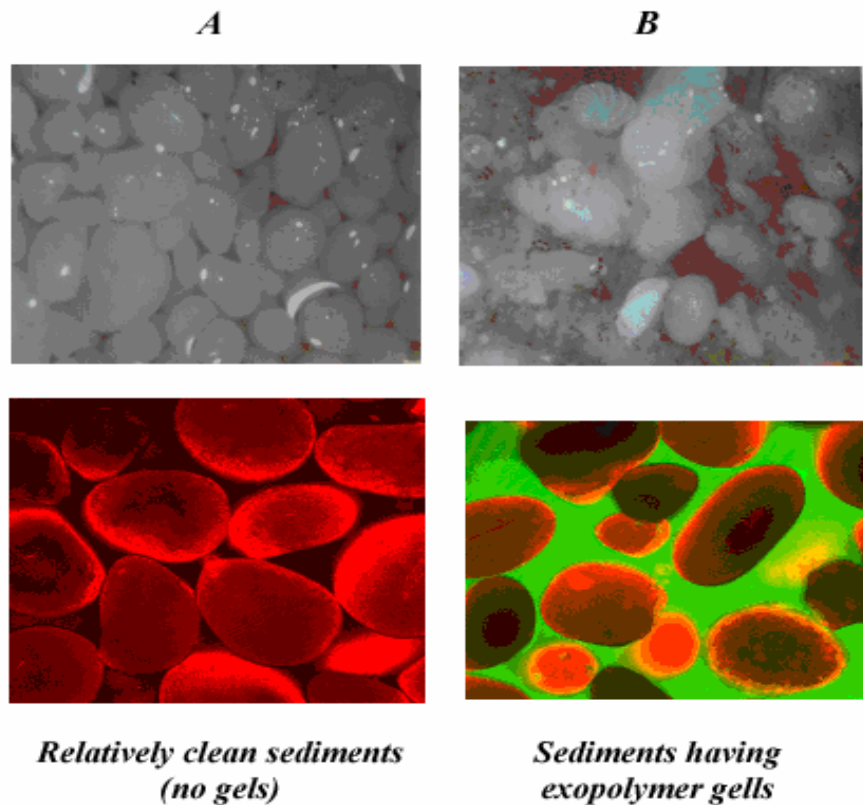
**Fig. 2. Spectral Reflectance by Exopolymer-Coated Sediments: Coating sediments with exopolymer decreases the magnitude of sediment reflectance, compared with uncoated (control) sediments.**

Decreases in reflectance are apparently not due to absorbance by exopolymers (Fig.3), which are relatively translucent over the visible range (400 – 700 nm).

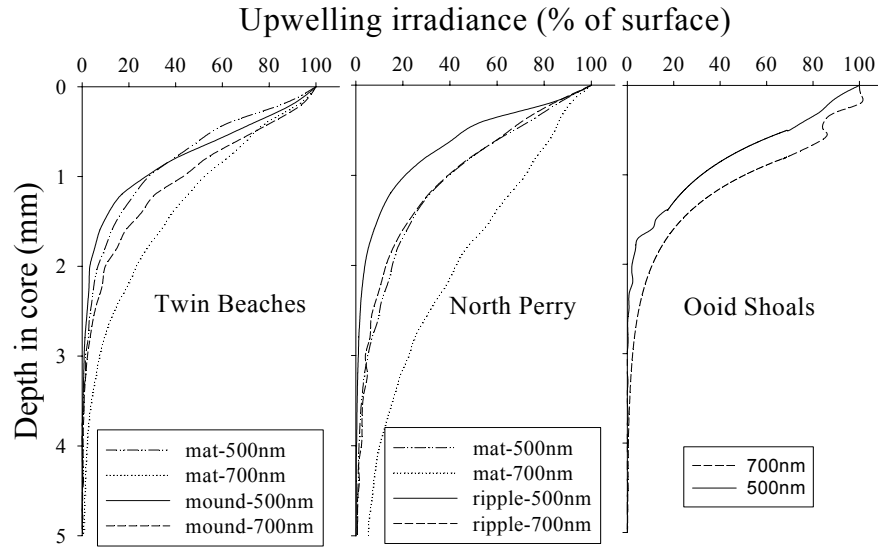


**Fig. 3. Light Absorbance by Exopolymers:** Natural exopolymers and artificial (alginate) polymers exhibit relatively low absorbance over the visible light range. Hence, Exopolymers act as an “optical lense” allowing more light to enter sediments.

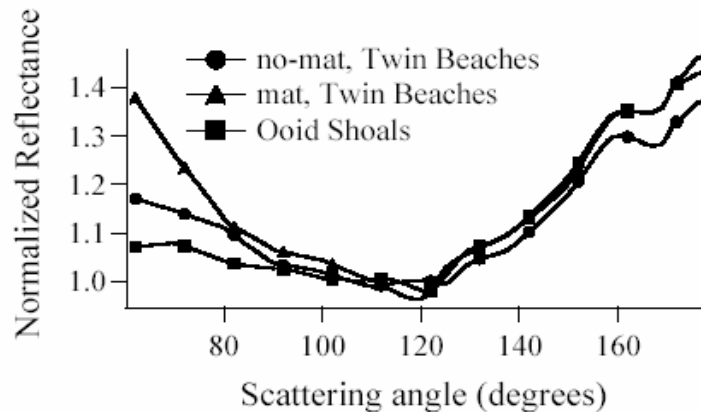
The relatively translucent nature of microbial exopolymers (Fig. 3) coupled to their ability to physically spread apart sediment grains, when in gel form (Fig. 4) allow a deeper penetration of light into sediments. This was measured (M. Allison) using optical probes (Fig. 5).



**Fig. 4. Spreading of sediment grains by exopolymer “gels” (B):** The presence of exopolymer gels accomplishes a physical spreading of sediment grains, relative to one another; when observed using light (upper) and confocal (lower) microscopy.



**Fig. 5. Presence of exopolymer-laden “mats” allows a deeper penetration of light into sediments (courtesy M. Allison)**



**Fig. 6. Presence of exopolymer gels in (mat) sediments increases forward scattering of light, relative to backscatter.**

The net effect is that the presence of high concentration exopolymers (present in microbial mats) serves to spread sediment grains apart, and channel light “into” sediments, rather than reflecting light off of the surface of sediments. This results in a relative decrease in the magnitude of reflectance (across all visible light wavelengths) due to the presence of microbial biofilms and their exopolymers.

## IMPACT/APPLICATION

Our previous results have shown that biofilms occur at virtually all sediment sites. When biofilms occur in highly abundance, they exert significant alterations on the optical spectra of sediment which is detectable using a range of different instrumentation. **We postulate that these effects may be detectable from remote sensing distances.** Biofilms (e.g., a diatom mat) cause a reduced overall spectral reflectance. This reduction results specifically from the exopolymer fraction (not the cells) of the biofilm. The exopolymer directly enhance forward scattering, but also change the relative spacing of sediment grains when biofilm mats are present. These two combined effects result in reflectance reductions of approx. 20%. We also have found that the fluorescence signatures of a surface may be “quenched” by the presence of a biofilm community of just 20 to 40 um thickness on that surface. Our results show that even “microscopic” microbial biofilms on sediments, which are not readily obvious, may exert strong effects on optical profiling of sediments.

## TRANSITIONS

The close coordination of “Sediment group” CoBOP personnel has provided a strong and unique dimension to our work. The past co-ordinated field experiments with other members of the Sediment Group in multi-investigator field experiments have been highly successful.

## RELATED PROJECTS

Below I list ongoing work in conjunction with CoBOP Biofilm project. Dr. R. P. Reid and myself are beginning a five year project sponsored by the National Science Foundation (NSF) addressing the carbonate sediments of “Marine Stomatolites” in the Bahamas. Stomatolites are sediment structures produced by bacteria. Their growth appears to depend on biofilm processes and light distributions (photosynthesis). Therefore, the data acquired from this project will be closely paired with results of our ongoing CoBOP studies.

## PUBLICATIONS

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**Decho, A.W.** and T. Kawaguchi. 1999. Confocal Imaging of in situ natural microbial communities and their extracellular polymeric secretions using Nanoplast resin. *BioTechniques* 27: 1246-1252.

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Kawaguchi, T. and **A.W. Decho**. 2002. A laboratory investigation of cyanobacterial extracellular polymeric secretions in influencing CaCO<sub>3</sub> polymorphism. *Journal of Crystal Growth* 240: 230-235.

Kawaguchi, T. and **A.W. Decho**. 2002. Isolation and Biochemical characterization of extracellular polymers (EPS) and their inhibitory effect on CaCO<sub>3</sub> precipitation. *Preparative Biochemistry and BioTechnology* 32: 51-63.

Kawaguchi, T. and **A.W. Decho**. 2002. In situ analysis of carboxyl- and sulfhydryl-groups of extracellular polymeric secretions by confocal scanning microscopy. *Analytical BioChemistry* 304: 266-267.

**Decho, A.W.**, T. Kawaguchi, M.A. Allison, E. Louchard, R.P. Reid, C. Stephens, K.J. Voss, R.A. Wheatcroft, B.B. Taylor. (in press) Sediment properties influencing upwelling spectral reflectance signatures: the “biofilm gel” effect. *Limnology and Oceanography*.

#### **Submitted Manuscripts:**

Kawaguchi, T., H. Al-Sayegh, **A.W. Decho**. (in review) Development of an indirect competitive enzyme-linked immunosorbent assay to detect extracellular substances (EPS) . *Journal of Immunoassays and Immunochemistry*.

**Decho, A.W.**, E. Louchard, K.J. Voss, M. Allison, C. Stephens, T. Kawaguchi, R.P. Reid, C.H. Mazel. (in review) Biofilm polymers act as an “optical lense” to trap visible light for photosynthesis through enhanced forward scattering. *Proc. Natl. Acad. Sciences*.